EXHIBIT I

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407. *Sulphates of Monosaccharides and Derivatives. Part IV.*[1] *Galactose 4-Sulphate.*

By J. R. Turvey and T. P. Williams.

D-Galactose 4-(sodium sulphate) has been synthesised by two definitive routes. Its properties are compared with those of D-galactose 6-(sodium sulphate).

It has long been recognised that various polysaccharides of red algæ contain residues of D-galactose, many of which bear sulphuric acid ester groupings.[2] Evidence from methylation and treatment with alkali suggests that some of these sulphate groups are situated at position 4 of the galactose units.[3] Despite this evidence, galactose 4-sulphate has neither been isolated from hydrolysis products of these polysaccharides nor been synthesised by chemical means. We now report definitive syntheses of this sugar sulphate and some of its properties.

Two similar routes were followed, both somewhat analogous to that used in the synthesis of 4-*O*-methanesulphonylgalactose.[4] In the first route, methyl 4,6-*O*-benzylidene-β-D-galactopyranoside was benzylated, to give the 2,3-di-*O*-benzyl derivative. After removal of the benzylidene group (acid–acetone), and subsequent triphenylmethylation, the resulting 2,3-di-*O*-benzyl-6-*O*-triphenylmethylgalactoside was sulphated at position 4 by use of pyridine–sulphur trioxide. This reaction required longer treatment with the sulphating reagent than is usual,[5] presumably owing to the axial orientation of the 4-hydroxyl group when the sugar derivative is in the favoured chair conformation. Removal of the blocking groups (benzyl and triphenylmethyl) by catalytic hydrogenation also proved difficult. It is evident that the ionic sulphate group interferes with the hydrogenation since the non-sulphated derivative was readily converted into methyl β-D-galactoside in high yield. Several repetitions of the hydrogenation with a palladium catalyst partly removed the protecting groups and acid-hydrolysis of the product gave a mixture of galactose and galactose 4-sulphate. The latter was isolated as the sodium salt in low yield by chromatography on thick filter paper.

A somewhat better yield was obtained by the alternative route, which followed the same steps as in the first method but in which benzyl β-D-galactopyranoside replaced the methyl galactoside. In this case, several hydrogenations (at 5 atm.) of the sulphated derivative gave a mixture from which the 4-sulphate was isolated. The two 4-sulphate preparations had similar optical rotations and were indistinguishable by chromatography or electrophoresis. Some properties of the synthetic sugar sulphate are listed in the Table and compared with those of galactose 6-sulphate.

Properties of galactose 4- and 6-(sodium sulphate).

Sugar sulphate	$[\alpha]_D$	R_F in solvents * (*a*)	(*b*)	(*c*)	M_G †
Galactose 4-(sodium sulphate)	+58·4°, +56·7°	0·27	0·28	2·09	1·04
Galactose 6-(sodium sulphate)	+47°	0·13	0·15	1·97	1·32

* (*a*) Butan-1-ol–ethanol–water (5 : 1 : 4, by vol.); (*b*) ethyl acetate–acetic acid–water (6 : 3 : 2, by vol.); (*c*) butan-1-ol–ethanol–water (3 : 1 : 1, by vol.) containing 3% (w/v) of cetylpyridinium chloride. † In 0·1*M*-borate buffer (pH 10·0).

The evidence on which the sulphate group is assigned to the 4-position, apart from the step-wise method of synthesis, is as follows. (i) The electrophoretic mobility, M_G (1·04), in borate buffer is less than that of galactose 6-, glucose 6-, or glucose 3-sulphate which

[1] Part III, *J.*, 1961, 2935.
[2] Hirst, *Proc. Chem. Soc.*, 1958, 177.
[3] Johnston and Percival, *J.*, 1950, 1994; Percival and Dewar, *J.*, 1947, 1622.
[4] Muller, Mocis, and Verner, *Ber.*, 1939, **72**, 745.
[5] Peat, Turvey, Clancy, and Williams, *J.*, 1960, 4761. – (Part I)

have M_6 values, 1·32, 1·26, and 1·27, respectively. Substituents on the 4-position of glucose or galactose are known to decrease the electrophoretic mobilities in borate buffer.[6] (ii) The infrared spectrum of each specimen showed an absorption peak at 850 cm.$^{-1}$ and no peak in the region 820—830 cm.$^{-1}$ In the latter region, peaks are shown by galactose 6- and glucose 3-sulphate. The occurrence of a peak at 850 cm.$^{-1}$ in the spectra of various galactan sulphates has been ascribed to the presence of an axially oriented 4-sulphate group in the galactose residues.[7] (iii) On periodate oxidation in unbuffered solution, the sulphate rapidly consumed 2 mol. of periodate, liberating 1 mol. of formic acid. Thereafter it underwent slow further oxidation until more than 5 mol. of periodate had been consumed. This behaviour is expected of a 4-*O*-substituted aldopyranose, which would be oxidised rapidly to a 2-*O*-substituted 3-*O*-formyltetrose, further oxidation then being dependent on the slow hydrolysis of the formyl ester.

Experimental

General Methods.—The general procedures for sulphation of sugar derivatives and paper chromatography of sugar sulphates have been described.[5] Preparative paper chromatography of sugar sulphates was carried out on Whatman 3MM paper with the freshly prepared solvent ethyl acetate–acetic acid–water (6 : 3 : 2, by vol.). Guide strips were sprayed with *p*-anisidine hydrochloride reagent.[8] With this reagent, galactose 4-sulphate gives a yellow spot, which fluoresces strongly in ultraviolet light, whereas galactose 6-sulphate gives a brown, weakly fluorescent spot. Paper electrophoresis was carried out in 0·1*M*-borate buffer (pH 10·0) at a potential gradient of 40 v per cm. for 1 hr. All solutions were evaporated under diminished pressure.

Galactose 4-(Sodium Sulphate) (First Route).—Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside. Methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (45 g.; m. p. 201—202°, $[\alpha]_D$ −36·8°; lit.,[9] m. p. 200°, $[\alpha]_D$ −35·1°) was treated with potassium hydroxide (40 g.) and benzyl chloride (150 ml.) according to the method of Dennison and McGilvray,[10] to give the *2,3-di-O-benzyl derivative* (90%), m. p. 133—134°, $[\alpha]_D^{20}$ +46·2° (*c* 0·3 in $CHCl_3$) (Found: C, 72·6; H, 6·35. $C_{34}H_{36}O_6$ requires C, 72·7; H, 6·55%).

Methyl 2,3-di-O-benzyl-β-D-galactopyranoside. Removal of the benzylidene grouping was attempted by heating the preceding product (60 g.) under reflux with 12*N*-hydrochloric acid (2·6 ml.) in acetone (900 ml.) and water (100 ml.) for 3 hr. After the mixture had been neutralised and evaporated to dryness, the product was dissolved in ethanol but some starting material crystallised. The ethanol solution was therefore evaporated to dryness and then refluxed for 2 hr. in a mixture of 0·25*N*-hydrochloric acid (160 ml.) and acetone (700 ml.). Neutralisation and evaporation gave the *dibenzyl ether* which was crystallised from ether–light petroleum (b. p. 40—60°) to give an almost theoretical yield of fine needles, m. p. 71—71·5°, $[\alpha]_D^{20}$ −1·4° (*c* 0·4 in $CHCl_3$) (Found: C, 67·0; H, 6·75. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0%).

Methyl 2,3-di-O-benzyl-6-O-triphenylmethyl-β-D-galactopyranoside. The dibenzyl ether (20 g.) in pyridine (350 ml.) was heated at 100° for 5 hr. with triphenylmethyl chloride (15 g.). The mixture was then cooled to 0° and poured into iced water (1 l.) containing an excess of sodium hydrogen carbonate. The syrup which separated solidified in 5 min. and was then extracted with chloroform. The extract, dried and evaporated to dryness, was freed from triphenylmethanol by being dissolved in hot ethanol to which water was added to a faint turbidity. On cooling, the *triphenylmethyl ether* separated and was recrystallised from ether–light petroleum (b. p. 40—60°), giving needles (18%), m. p. 103—104°, $[\alpha]_D^{20}$ −13·2° (*c* 0·4 in $CHCl_3$) (Found: C, 77·5; H, 6·7. $C_{58}H_{56}O_6$ requires C, 77·9; H, 6·55%).

Sulphation. The triphenylmethyl ether (4·5 g.) was treated[5] with pyridine–sulphur trioxide at 65° for 21 hr. and, after being cooled, the mixture was stirred for 2 hr. in 50% aqueous ethanol. The solution was then adjusted to pH 9 with saturated aqueous barium hydroxide, and the precipitate removed on the centrifuge. The supernatant solution was distilled to

[6] Bouveng and Lindberg, *Acta Chem. Scand.*, 1956, **10**, 1283.
[7] Orr, *Biochim. Biophys. Acta*, 1954, **14**, 173; Lloyd, Dodgson, Price, and Rose, *ibid.*, 1961, **46**, 108.
[8] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[9] Oldham and Pell, *J. Amer. Chem. Soc.*, 1938, **60**, 323.
[10] Dennison and McGilvray, *J.*, 1951, 1616.

remove ethanol and pyridine, water being added to maintain the volume. The crude product, which had separated, was washed with water and dissolved in ether, and the solution was dried and concentrated to a syrup. This was precipitated from ether (500 ml.) by addition of light petroleum (b. p. 40—60°; 500 ml.), non-sulphated material remaining in solution. The white, amorphous product, *methyl* 2,3-*di*-O-*benzyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside* 4-(*barium sulphate*) (4·9 g.) had $[\alpha]_D^{19}$ +15·1° (*c* 0·3 in $CHCl_3$) [Found: Ba, 8·8; S, 4·1. $(C_{10}H_{23}O_9S)_2Ba$ requires Ba, 9·0; S, 4·2%]. Hydrogenation of the barium salt was attempted with Raney nickel (at 20° and at 60°), platinum oxide, and palladium–charcoal, but only the last-named effected appreciable reduction. The barium salt (2 g.) was therefore hydrogenated at 1 atm. in 80% ethanol (75 ml.) over 5% palladium–charcoal [11] (2 g.) for 16 hr. The catalyst was removed and was washed with 80% ethanol, and the combined filtrate and washings were evaporated to dryness. The hydrogenation was then repeated with fresh catalyst (2 g.). The product was extracted with water, and the extracted material (880 mg.) examined by paper chromatography. The main product was still only partly hydrogenated but traces of a galactoside sulphate were also present. The mixture (310 mg.) was treated with the ion-exchanger Zeo-Karb 225 (Na^+ form), and the resulting sodium salt was heated in 0·1N-sulphuric acid for 1 hr. at 100°. Neutralisation with barium hydroxide, removal of insoluble salts on the centrifuge, and evaporation gave a product (270 mg.) containing galactose, galactose monosulphate, and methyl β-galactoside. The *galactose* 4-(*sodium sulphate*) (sample 1) was separated by chromatography on thick paper. The yield was 52 mg., and $[\alpha]_D^{16}$ +58·4° (*c* 0·9 in H_2O) (Found: S, 11·5. $C_6H_{11}NaO_9S$ requires S, 11·4%). The sulphate was readily distinguished from galactose 6-(sodium sulphate) by its R_F values in the solvent systems named in the Table (for (*c*) see Rees [12]), as well as by its M_G value in borate buffer (see Table).

Galactose 4-(*Sodium Sulphate*) (*Second Route*).—*Benzyl* 4,6-O-*benzylidene*-β-D-*galactopyranoside*. Benzyl β-D-galactopyranoside, prepared from 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-galactose and benzyl alcohol by Fischer and Helferich's method,[13] had m. p. 99—100°, $[\alpha]_D^{16}$ −27·2° (lit.,[14] m. p. 100—101°, $[\alpha]_D$ −25·05°). The galactoside (50 g.) was shaken with zinc chloride and benzaldehyde, and the product isolated by Klemer's method.[15] *Benzyl* 4,6-O-*benzylidene*-β-D-*galactoside* crystallised from acetone as needles (60 g.), m. p. 209—210°, $[\alpha]_D^{20}$ −62·4° (*c* 0·7 in $CHCl_3$) (Found: C, 67·0; H, 6·1. $C_{20}H_{22}O_6$ requires C, 67·0; H, 6·2%).

Benzyl 2,3-*di*-O-*benzyl*-4,6-O-*benzylidene*-β-D-*galactopyranoside*. The preceding product (50 g.) was stirred at 100° under anhydrous conditions with powdered potassium hydroxide (50 g.) and benzyl chloride (80 ml.). After 1 hr. the mixture solidified and more benzyl chloride (25 ml.) was therefore added. After being stirred for a further 2 hr. at 100°, the mixture was cooled and iced water (500 ml.) was stirred in. The solid was removed by filtration and washed with water until no longer alkaline, then washed with light petroleum and dried. Recrystallisation from acetone gave needles of the 2,3-*dibenzyl ether* (68·9 g.), m. p. 169·5—170·5°, $[\alpha]_D^{20}$ −2·3° (*c* 0·8 in $CHCl_3$) (Found: C, 76·6; H, 6·5. $C_{34}H_{34}O_6$ requires C, 75·8; H, 6·4%).

Benzyl 2,3-*di*-O-*benzyl*-D-*galactopyranoside*. The last-mentioned dibenzyl ether (50 g.) was hydrolysed by Bell and Lorber's method.[16] The *product* (39 g.), recrystallised from acetone–light petroleum (b. p. 40—60°), had m. p. 116—117°, $[\alpha]_D^{20}$ −17·0° (*c* 0·6 in $CHCl_3$) (Found: C, 72·3; H, 6·5. $C_{27}H_{30}O_6$ requires C, 72·0; H, 6·7%).

Benzyl 2,3-*di*-O-*benzyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside*. The last product (32 g.) in dry pyridine (300 ml.) was heated at 100° for 1 hr. with triphenylmethyl chloride (28 g.), then cooled and added to iced water (2·5 l.). After 2 hr., the product was removed by filtration and dissolved in chloroform. The solution was washed with aqueous sodium carbonate and then with water and dried ($CaCl_2$). Evaporation of the solution gave an amorphous powder, which did not crystallise. The 6-O-*triphenylmethyl ether* (31 g.) had $[\alpha]_D^{20}$ −29·7° (*c* 0·4 in $CHCl_3$) (Found: C, 79·4; H, 6·3. $C_{46}H_{42}O_6$ requires C, 79·7; H, 6·4%).

Sulphation. The triphenylmethyl ether (5 g.) was sulphated with pyridine–sulphur trioxide (5 g.) for 24 hr. at 65—70° and the product isolated as described above, giving an amorphous sulphated derivative (3·5 g.; barium salt). This product (1 g.) in 80% ethanol (75 ml.) was

[11] *Org. Synth.*, 1946, **26**, 78.
[12] Rees, *Nature*, 1960, **185**, 309.
[13] Fischer and Helferich, *Annalen*, 1911, **383**, 68.
[14] Bourquelot, Hérissey, and Bridel, *Compt. rend.*, 1913, **156**, 330.
[15] Klemer, *Chem. Ber.*, 1959, **92**, 222.
[16] Bell and Lorber, *J.*, 1940, 453.

hydrogenated at 5 atm. for 13 hr. over 5% palladium–charcoal [11] (1 g.) a total of four times. The final syrup was extracted with water, and the extracts were evaporated to a syrup (253 mg.) containing galactose and its 4-(barium sulphate). The galactose sulphate was separated by chromatography on thick paper and converted into the sodium salt with Zeo-Karb 225 (Na^+ form). The galactose 4-(sodium sulphate) (sample 2, 87 mg.) had $[\alpha]_D^{18}$ +56·7° (*c* 0·15 in H_2O) (Found: S, 11·6. Calc. for $C_6H_{11}NaO_9S$: S, 11·4%). This product was identical in R_F and M_G values with that obtained by the first method.

Periodate Oxidation.—Galactose 4-(sodium sulphate) (sample 2, 1·8 mg.) was oxidised in 0·01*M*-sodium metaperiodate (3 ml.) in the dark at 35° and the periodate consumed measured by the method of Aspinall and Ferrier: [17]

Time (hr.)	0·25	0·55	1·05	1·75	2·75	4·25	7·5
Periodate consumed (mol.)	0·58	1·28	1·68	1·96	2·01	2·05	2·12

After 4 hr. a portion (1 ml.) of the mixture was withdrawn for estimation of formic acid liberated.[18] The amount found was 0·95 mol. Sample 1, similarly oxidised, gave the following results:

Time (hr.)	0·5	2·0	9·0	25	98	312
Periodate consumed (mol.)	1·1	1·7	1·98	2·56	4·33	5·43

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Department of Chemistry, University College of North Wales,
Bangor, Caernarvonshire. [*Received, January 29th*, 1962.]

[17] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[18] Peat, Whelan, and Turvey, *J.*, 1956, 2317.

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408. *Dissolution of Metals and Alloys. Part II.*[1] *The Dissolution of Iron, Cobalt, and Copper, and of Some Nickel–Copper Alloys in Solutions of Sodium Persulphate.*

By G. C. Bond and G. Webb.

Iron, copper, and cobalt foils dissolve in solutions of sodium persulphate near room temperature, their reactivity decreasing in the order listed. Rates free from transport-limitation resulted on use of M-solutions and rotation of the foils at 500 r.p.m., and the following activation energies were found: Fe, 6·5; Co, 11·9; Cu, 11·2 kcal. $mole^{-1}$. Rates of dissolution of the three metals have been measured over at least the concentration range 0·1—1M, with rates of rotation of zero, 250, and 500 r.p.m. at three temperatures, and the results have been interpreted.

Nickel is unreactive.

The activation energies for the transport-free dissolution of nickel–copper alloys fall with increasing nickel content to a limiting value of 7·2 kcal. $mole^{-1}$ which holds for nickel contents between 40 and 90%. The rates vary irrationally from one alloy to another owing to development of different degrees of surface roughness. The rate-limiting step, for both alloys and metals, is probably the transfer of an electron therefrom to a persulphate ion.

In Part I [1] we described preliminary experiments on the dissolution of copper in solutions containing persulphate ion: these were performed to assess whether this reaction could be used for studying the role of electronic factors in dissolutions. It was predicted that rates free from transport-limitation should be obtainable near room temperature, at high concentrations of persulphate ion (~ M), and with rapid stirring (500 r.p.m.).

[1] Part I, Bond, Hill, and Tennison, *J.*, 1959, 33.